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To:	Name:	Mark Farley
	Company:	
	Fax Number:	212 391 0525
	Voice Phone:	212 278 0418
From:	Name:	Anne Holleran
	Official Fax Number:	(703) 872-9306
	Official After Final Fax Number:	(703) 872-9307
	Voice Phone:	703-308-8892

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Fax Notes:

Proposed examiner's amendment for 08/196,154. Please note that claim 126 needs to be cancelled because it has the same scope as claim 129. Also, because of amendment to 08/477,147, this case needs a T.D. over 08/477,147.

My telephone number is 571-272-0833. Anne Holleran

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EXHIBIT A
Applicants: Livingston et al.
U.S. Serial No.: 08/475,784
Filed: June 7, 1995

Application/Control Number: 08/196,154
Art Unit: 1642

PROPOSED EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with xxx on xxx.

The application has been amended as follows:

In the specification:

at page 38, line 13, after "(Kensil et al. 1991)", the following was added:

Coursely chopped Q. saponaria bark [approximately 1 cm square, obtained from Hauser Chemicals, Boulder, CO] was stirred with 10 ml of water/g of bark at room termperature for 1 h. The extract was centrifuged and the supernatant containing the solubilized saponins was saved. The extraction step was repeated on the bark pellet and the two supernatants were pooled. To remove nonsaponin components, the supernatant pool was lyophilized, redissolved in 40 mM acetic acid in water at a concentration of 250 mg/ml (w:v) and either chromatographed through Sephadex G-50 (medium, Pharmacia, Piscataway, NJ) in 40 mM acetic acid with the hemolytic activity localized in the void volume fraction, or dialyzed against 40 mM acetic acid with the hemolytic activity retained by the dialysis membrane. The hemolytic fraction was lyophilized and redissolved at a concentration of 200 mg/ml in 40 mM acetic acid in

chloroform/methanol/water (62/32/6, v/v/v): 1 g of this fraction was applied to Silica Lichroprep (E.M. Science, Gibbston, NJ: 40 to 63 µm particle size, 2.5 cm I.D. x 20 cm height) and eluted isocratically in the solvent used to solubilize the saponins. The elution of saponins was monitored by carbohydrate assay. Fractions containing he saponins of interest were identified by reverse phase TLC with visualization with Bial's reagent (Sigma, ST. Louis, M)) pooled individually, and rotavapped to dryness. The fractions from the silica chromatography were then redissolved in 40 mM acetic acid in 50% methanol and loaded on a semipreparative HPLC column (Vydac C_4, 5µm particle size, 3000 nm pore size, 10 mm I.D. X 25 cm length). Saponin peaks detectd by absorbance at 214 nm were eluted by using a methanol gradient at a flow rate of 4 ml/min and individually rotavapped to dryness. Purity of saponins was assessed by analytic HPLC (Vydac C_4, 5µ particle size, 3000 nm pore size, 4.6 mm I.D. x 25 cm length) with a gradient of 0.1% TFA in acetonitrile. QS-21 is defined as the adjvant active reverse phase HPLC fraction 21 from Q. Saponaria bark extract.

In the claims:

Claim 126 was canceled.

Claim 119. A composition which comprises:

a) a conjugate of (i) a GM2 ganglioside derivative [which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base], wherein the GM2 ganglioside derivative is a GM2 ganglioside cleaved

with ozone, and wherein an aldehyde group is introduced at the C4 position of the sphingosine portion of the GM2 ganglioside, and (ii) Keyhole Limpet Hemocyanin[;], wherein the GM2 ganglioside derivative is covalently bound to the Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the sphingosine base and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin;

b) QS-21[, a saponin derivable from the bark of a Quillaja saponaria Molina tree]; and

c) a pharmaceutically acceptable carrier;

wherein the amount of the conjugated GM2 ganglioside derivative is an amount between about 1 μg and about 200 μg, the amount of [the saponin] QS-21 is an amount between about 10 μg and about 200 μg, and the GM2: Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, the relative amounts of such conjugate and [such saponin] QS-21 being effective to stimulate or enhance production in a subject of an antibody to GM2[,]

[wherein in the conjugate the ganglioside derivative is covalently bound to the Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin].

Claim 127. The composition of 119 wherein the amount of the [saponin] QS-21 is about 200 μg.

Claim 129 The composition of claim 119 which comprises:

a) a conjugate of (i) a GM2 ganglioside derivative [which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base], wherein the GM2 ganglioside derivative is a GM2 ganglioside cleaved with ozone, and wherein an aldehyde group is introduced at the C4 position of the sphingosine portion of the GM2 ganglioside, and (ii) Keyhole Limpet Hemocyanin[;], wherein the GM2 ganglioside derivative is covalently bound to the Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the sphingosine base and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin;

b) QS-21[, a saponin derivable from the bark of a Quillaja saponaria Molina tree]; and

c) a pharmaceutically acceptable carrier;

wherein the conjugated GM2 ganglioside derivative is present in an amount between about 1 μg and about 200 μg, the amount of [the saponin] QS-21 is about 100 μg and the GM2: Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, the relative amounts of such conjugate and [such saponin] QS-21 is effective to stimulate or enhance production in a subject of an antibody to GM2[,]

[wherein in the conjugate the ganglioside derivative is covalently bound to the Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin].

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Claim 131. A method of stimulating or enhancing production of an antibody directed to GM2 in a subject which comprises administering to the subject an effective amount of a composition which comprises:

a) a conjugate of (i) a GM2 ganglioside derivative [which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base], wherein the GM2 ganglioside derivative is a GM2 ganglioside cleaved with ozone, and wherein an aldehyde group is introduced at the C4 position of the sphingosine portion of the GM2 ganglioside, and (ii) Keyhole Limpet Hemocyanin[;], wherein the GM2 ganglioside derivative is covalently bound to the Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the sphingosine base and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin;

b) QS-21[, a saponin derivable from the bark of a Quillaja saponaria Molina tree]; and

c) a pharmaceutically acceptable carrier;

wherein the amount of the conjugated GM2 ganglioside derivative is present in an amount between about 1 μg and about 200 μg, the amount of [the saponin] QS-21 is amount between about 10 μg and about 200 μg, and the GM2: Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, the relative amounts of such conjugate and [such saponin] QS-21 is effective to stimulate or enhance production in a subject of an antibody to GM2[,]

[wherein in the conjugate the ganglioside derivative is covalently bound to the Keyhole Limpet Hemocyanin by a stable amine bond between the C-4

carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin] so as to thereby stimulate or enhance production in said subject of [the antibody] antibodies directed to GM2.

Claim 132. A method of treating a human subject which comprises administering to the subject an effective amount of a composition which comprises:

a) a conjugate of (i) a GM2 ganglioside derivative [which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base], wherein the GM2 ganglioside derivative is a GM2 ganglioside cleaved with ozone, and wherein an aldehyde group is introduced at the C4 position of the sphingosine portion of the GM2 ganglioside, and (ii) Keyhole Limpet Hemocyanin[;], wherein the GM2 ganglioside derivative is covalently bound to the Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the sphingosine base and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin;

b) QS-21[, a saponin derivable from the bark of a Quillaja saponaria Molina tree]; and

c) a pharmaceutically acceptable carrier;

wherein the amount of the conjugated GM2 ganglioside derivative is present in an amount between about 1 μg and about 200 μg, the amount of [the saponin] QS-21 is amount between about 10 μg and about 200 μg, and the GM2: Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, the relative amounts of such conjugate

and [such saponin] QS-21 is effective to stimulate or enhance production in a subject of an antibody to GM2[,]

[wherein in the conjugate the ganglioside derivative is covalently bound to the Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin] so as to stimulate or enhance production in the subject of [the antibody] antibodies to GM2 and thereby treat the subject.

Claim 142. The method of claim 141, wherein the conjugate and the [saponin] QS-21 are mixed on the day of administration to the subject.